

Atty. Dkt. No. 0709.010.0002
United States Serial No. 10/721,091

AMENDMENTS TO THE SPECIFICATION

Applicants respectfully request that the specification of the current application be amended according to the following.

Please delete paragraphs 0031 and 0032 and replace with the following paragraphs.

[0031] The fusion proteins of the current invention also comprise a labeling moiety. A labeling moiety, as used herein, is intended to mean a chemical compound or ion that possesses or comes to possess a detectable non-radioactive signal. Examples of labeling moieties include, but are not limited to, transition metals, lanthanide ions and other chemical compounds. The non-radioactive signal includes, but is not limited to, fluorescence, phosphorescence, bioluminescence and chemiluminescence. In one embodiment, the labeling moiety is a fluorophore selected from the group consisting of fluorescein, coumarins, rhodamines, 5-TMRIA (tetramethylrhodamine-5-iodoacetamide), Quantum Red™, Texas Red™, [[Cy3,]] Cy3™, N-((2-iodoacetoxy)ethyl)-N-methylamino-7-nitrobenzoxadiazole (IANBD), 6-acryloyl-2-dimethylaminonaphthalene (acrylodan), pyrene, Lucifer Yellow, [[Cy5,]] Cy5™, Dapoxyl® (2-bromoacetamidoethyl)sulfonamide, (N-(4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene-2-yl)iodoacetamide (Bodipy507/545 IA), (BODIPY® 507/545 IA), N-(4,4-difluoro-5,7-diphenyl-4-bora-3a,4a-diaza-s-indacene-3-propionyl)-N'-iodoacetylthylenediamine (BODIPY® 530/550 IA), 5-((((2-iodoacetyl)amino)ethyl) amino)naphthalene-1-sulfonic acid (1,5-IAEDANS), and carboxy-X-rhodamine, 5/6-iodoacetamide (XRIA 5,6). Other luminescent labeling moieties include lanthanides such as europium (Eu³⁺) and terbium (Tb³⁺), as well as metal-ligand complexes of ruthenium [Ru(II)], rhenium [Re(I)], or osmium [Os(II)], typically in complexes with diimine ligands such as phenanthroline.

[0032] In particular, the fluorophore labeling moiety can be fluorescein, acrylodan, rhodamine, BODIPY, BODIPY®, eosin, pyrene, acridine orange, PyMPO, ~~alexa fluor 488, alexa fluor 532, alexa fluor 546, alexa fluor 568, alexa fluor 594, alexa fluor 555, alexa fluor 633, alexa fluor 647, alexa fluor 660, or alexa fluor 680.~~ Alexa Fluor™ 488, Alexa Fluor™ 532, Alexa

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Fluor™ 546, Alexa Fluor™ 568, Alexa Fluor™ 594, Alexa Fluor™ 555, Alexa Fluor™ 633, Alexa Fluor™ 647, Alexa Fluor™ 660, or Alexa Fluor™ 680. More particularly, the labeling moiety may be acrylodan. In another embodiment, the labeling moiety is an electrochemical moiety such that a change in the environment of this labeling moiety will change the redox state of the moiety.

Please delete paragraph 0037 and replace with the following paragraph.

[0037] In one embodiment of the current invention, device in which the functional GGBP(s) may be immobilized is a sensor attached to a collection of optical fibers. The fiber used in this embodiment may be a bifurcated fiber optic bundle. In one particular embodiment, the fiber optic contains six outer fibers arranged around a central fiber. The six fibers can be used as the excitation conduit and the central fiber as the detection conduit. These collection optics may also include additional fibers and/or lenses. The fiber can be polished, and then medical grade glue, or any other suitable adhesive, for example, Loctite 4011, Loctite™ 4011, can be applied to adhere the sensing element to one end of the fiber optic. The other end of the fiber bundle is connected to a fiber optic spectrophotometer. An LED at the appropriate wavelength (e.g., LS-450) can then be used and a fluorescence spectrometer can be used as a detector. Excitation sources may consist of, but are not limited to, for example arc lamps, laser diodes, or LEDs. Detectors may consist of, but are not limited to, for example, photodiodes, CCD chips, or photomultiplier tubes. A computer program, such as Ocean Optic OOIBase 32, may also be employed to trace the fluorescent emission.

Please delete paragraph 0070 and replace with the following paragraph.

[0070] Plasmid pTZ18R contains the MgLB gene from *E. coli* strain JM109. The GGBP gene was amplified from pTZ18R. The GGBP gene was ligated into the pQE70 plasmid to create a histidine-tagged protein that is wild-type in sequence, except for a lysine-to-arginine change at amino acid position 309, and the addition of a serine at amino acid position 310, before the six histidines at the C-terminus. The DsRed2 gene was amplified from (pDsRed2) and ligated to the

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N-terminus of the GGBP gene. A short three-alanine linker was engineered into the construct between the fluorescent protein and the histidine-tagged GGBP. Mutations of the GGBP and/or the fluorescent protein were generated in the construct by standard methods. For example, PCR was performed using primers that substitute codon(s) at or near the primary glucose contact sites. This removes the cysteine residue from the DsRed2 portion of the fusion so that when the fusion is fluorophore labeled the label will be site-specifically conjugated to GGBP only. All proteins were histidine-fusions and sequences were confirmed by sequencing. A representation of the dsRed2/GGBP fusion protein tetramer is shown in FIG. 2. This was created using coordinates from crystal structures of the two individual proteins (PDB ID's: 1GGX and 2GBP respectively).